**How the Butterfly Gets Its Spots**

**And what they tell us about fate**

**Susan Milius**

Paul Brakefield is a world authority on spots. His laboratory team delves into wide-ranging questions about the circles and dots on butterfly wings: What genes change the spots' size? Do different spots evolve separately or in concert? What kinds of spots wow the opposite sex? Brakefield argues that butterfly wings, particularly their freckles, offer science a rare opportunity. They're good for experiments in an unusually wide range of scientific disciplines, so researchers can combine insights and deepen their understanding of how evolution works.

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| SEE SPOT GROW. The buckeye butterfly's bold spots enabled researchers to discover that the location of the adults' bull's-eyes is determined while the insect is still a caterpillar. |

Several labs, including Brakefield's at the University of Leiden in the Netherlands, are illuminating the genetics of wing patterns. These scientists can take advantage of findings in a less picturesque but thoroughly studied insect—the fruit fly. One group is even making progress on creating transgenic butterflies with fluorescent proteins in their tissue.

Developmental biologists, too, find the butterfly wing a great subject. Its simplicity as a two-dimensional sheet, instead of a three-dimensional organ, makes analysis more straightforward than in most other systems.

The beauty of the butterfly to behavioral ecologists, says Brakefield, is that its wing spots, bands, and other splotches play dramatic roles in mating, outwitting predators, and other matters critical to the insect's evolution.

Eventually, butterfly scientists expect to trace the workings of evolution from the folds of a molecule to the breadth of a continent. They have been saying for years that the tale of evolution is written on the wings of a butterfly. Now, they're beginning to decipher it.

**Basic scales**

Most of the world's 17,000 butterfly species sport distinctive wings, some as patterned as embroidery samplers, some so iridescent that that a butterfly's bright flash at ground level has caught the eye of passengers in small airplanes overhead. This plethora of wing designs comes from tiny scales decorated by two sets of artists' tools. On the scales' surface, microscopic structures—some shaped like tiny Christmas trees—create color by playing tricks with light. However, analysis of their genetics lags far behind that of the other source of color, pigment-based wing displays.

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| SPOT LIGHTS. Glow that shows gene activity (top images) in tissue in a pupa's developing wing foreshadows adult wing-pattern elements (bottom images). Beldade, Brakefield |

Much as computer screens form images, butterfly wings use tiny, single-color flecks that add up to the big pattern. Each minuscule, colored scale comes from a single cell, which dies after it's made its pixel. The colors come from mixtures of pigments—flavonoids, melanins, and pterins—that are widespread among organisms.

Caterpillars already carry disks of future wing cells, including those that will lay down the colorful design. In the 1970s, H. Frederik Nijhout of Duke University in Durham, N.C., performed miniature transplant surgeries, switching around bits of caterpillar tissue to see when the fates were determined for various wing parts. He worked with the buckeye butterfly (*Precis coenia*), which has bold bull's-eye spots on its fore and hind wings. Nijhout showed that before the caterpillar retires into its chrysalis to transform into a butterfly, the position of the spots on the future wings has been established.

A milestone in the genetics of wing pattern came in the early 1990s when Sean Carroll of the University of Wisconsin–Madison and his colleagues searched for butterfly counterparts to the genes known to control formation and patterning of wings in fruit flies. They worked with an African species that also has big bull's-eyes, *Bicyclus anynana*. Most of the genes they tested played a role similar to their action in fruit flies. But then came a test of the *Distal-less* gene (*Dll*).

Carroll still remembers the Friday when one of his colleagues called him to look at the latest results. The team had finally found a gene that decorates a butterfly wing. In fruit flies, *Dll* influences limb formation, but in the caterpillar, *Dll* also defines the center point of each of the bull's-eyes on *B. anynana*'s wings.

Suddenly a world of possibilities opened for testing pattern genetics. "You only need a few days like that and you can put up with years of frustration," Carroll recalls.

**Variations on a gene**

Geneticists now know of several other genes that flicker on during formation of patterns such as the bull's-eye's rings. Still, *Dll* remains a favorite in genetics labs.

Patrícia Beldade, now at the University of California, Irvine, and her colleagues have tested whether there are variations in *Dll* that might provide raw material for natural selection, creating fitter and less-fit individuals that will prosper or fail as evolution grinds along.

Not every gene has to contribute to measurable variation among individuals, says Beldade. For example, extensive variants may not have evolved because a gene plays such a vital role that any alteration kills the organism. Beldade says that *Dll* did look valuable because it shows up with similar functions in insects that have followed divergent evolutionary paths. "Maybe you don't want to mess with it," she says.

To see whether *Dll* varies, Beldade and her Leiden colleagues worked with a laboratory colony of *B. anynana*. For nine generations, the scientists intervened in the insect's breeding to create a lineage with big bull's-eyes and another with small ones.

When Beldade analyzed various crosses of these lineages, she found specific forms of the gene associated with either large or small bull's-eyes. Variety in *Dll* itself or in a companion stretch of DNA powers spot variation, she and her colleagues reported in the Jan. 17, 2002 *Nature*.

In the real world, of course, a spot must be considered in the context of the entire organism. Just how the fate of one feature tugs at that of the others has intrigued Antónia Monteiro, now of the University at Buffalo, New York. Her lab is now developing transgenic butterflies with fluorescent pigments for future genetic tests.

In the early 1990s, she and her colleagues turned to the spots of *B. anynana* to explore the tangled fate of traits. She, too, began a butterfly-breeding program. She focused on maximizing the size of a particular wing bull's-eye. As that spot grew in succeeding generations, she found that other bull's-eyes enlarged, too.

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| UNCOUPLED. These wings of Bicyclus anynana at the end of an artificial breeding experiment show that it's possible to push the evolution of spots on the same wing in opposite directions. C. Brunetti, J. Selegue, Carroll |

Later, Beldade considered just how tightly the fates of all those spots are coupled. Maybe genes forced all of a butterfly's spots to stay in lockstep, either all getting larger or all dwindling. She and her colleagues selectively bred *B. anynana* to see if they could simultaneously drive the evolution of two spots on a wing in opposite directions. For 17 insect generations, the researchers selected for lineages according to mixed-and-matched spot size, such as a big front spot and a small rear spot.

"After a few generations, I knew it was possible," says Beldade. In the lineages that her team created, spot size at one location had indeed uncoupled from the size at the other, she and her colleagues reported in the April 25, 2002 *Nature* and the Oct. 29, 2002 *Proceedings of the National Academy of Sciences*.

These papers dashed the notion that butterfly-spot size represents an example of a trait that's evolved under such a strong constraint that some forms, such as one big spot and one little spot, never develop, according to Beldade. Other proposed examples of traits that can't evolve certain forms include the number of neck vertebrae in mammals—seemingly always seven—and the number of leg-bearing segments of centipedes—seemingly always odd. These examples have all been controversial.

**What wings say**

Does all this variation in spottiness make a difference to the butterflies? Brakefield and his Leiden colleague Casper Breuker decided to test how wing decoration affects the sex life of *B. anynana*.

The researchers set up cages and offered females a choice of two or three males whose spots the researchers had altered in different ways. Biologists have evidence suggesting that females of some animal species respond to how symmetrical a male is, but these female butterflies did not seem to show a preference for males with or without same-size spots on each side, the researchers reported in the June 22 *Proceedings of the Royal Society of London B*.

Likewise, the female butterflies failed to show any pattern of response to a male's wing size. They did respond to spot sizes, though, preferring the guys with big bull's-eyes.

Carol Boggs at Stanford University is exploring how the sexiness of wing pattern interacts with other butterfly concerns, like survival. She and Jacintha Ellers, now at the Institute of Ecological Science in Amsterdam, analyzed coloration of clouded sulphur butterflies (*Colias eriphyle*) in Colorado's Rocky Mountains. The sulfur-yellow background of the wings carries a smattering of dark spots near the edges.

Across their hind wings, females develop more black pigment than males do and so take on a smoky cast, Ellers and Boggs reported in the April 2002 *Evolution*. When the researchers checked butterflies living on mountainsides at elevations from about 1,800 meters to 2,900 m, they found that the darkening tends to intensify with elevation.

Boggs proposes an advantage of the smokiness. Darker wings can help a butterfly warm up and move faster by absorbing extra sunlight, she explains. Butterflies are at the mercy of their environment for body heat, so a heat bonus might bring important benefits-especially at cooler elevations. Females, which at times lug around weighty eggs, could find that dark-wing advantage of special importance.

The story is about to get more interesting, Boggs predicts. A report she and Ellers will publish soon in *Evolution* shows that males, regardless of location on the mountainsides, prefer lighter-winged females to darker ones, says Boggs. Too bad if lighter females suffer chilling disadvantages. The butterflies therefore present an example of a clash between what's good for survival and what's good for attracting a mate.

The study of evolution has turned up these clashes before. In most of these, such as the classic example of the peacock's tail, sexiness dominates although it's limited by such survival disadvantages as slower escape from predators. But Boggs suspects that the butterfly story will have an unusual ending because the female clouded sulphurs do darken as their home altitudes rise. Boggs says that she bets that the clouded sulphurs will provide an example of survival value dominating, limited by the demands of sex appeal.

That's just one of the evolutionary issues a scientist can test with butterflies, and Boggs says that plenty of others will appear in a 700-page tome on butterfly research that she and two of her colleagues edited for publication later this year.

The promise of the field reminds her of the words of 19th-century English naturalist-collector Henry W. Bates. He spent 11 years exploring the Amazon, and his haul of 14,000 species included many butterflies. He wrote that understanding their variety would unveil the forces driving variety in all life and that "the study of butterflies, creatures selected as the types of airiness and frivolity, instead of being despised will someday be valued as one of the most important branches of biological science."

**Fights on the Wing**

**If all butterflies do is flutter, how does the winner win?**

If you can't punch, kick, stab, shoot, bite, squash, or even touch some jerk, how can you fight him? Yet male butterflies of many species manage this feat all the time, says Darrell Kemp, now at Arizona State University in Tempe. Male butterflies disagree over the usual things—territories, females—yet fight duels that eventually send one contestant flying, all without physical contact.

To figure out this remarkably unbloody warfare, Kemp worked in Australia on the feisty *Hypolimnas bolina*, or common egg fly. Males compete relentlessly to claim a territory where unmated females are likely to waft by. A female only mates once, but males mate as often as possible. So, in a given part of a forest, all the males are ready to go, but "95 percent of all the females aren't receptive," says Kemp. "It's a scrap-fight over the remaining 5 percent."

When one male intrudes on another, the pair starts flying close to the ground in a circle the size of a dinner plate. Typically, one combatant flees in a few minutes, but battles can last a quarter hour. The departing male flaps off with an unusual gliding rhythm that Kemp suspects is a loser butterfly's submissive slink.

The best predictor of success turns out to be age, says Kemp. He found that males reach their competitive peak during the last third of their 3-month life span. "These older butterflies are run-down. Their wings are really torn. Usually, they don't have much in the way of energy reserves," he says. Yet they triumph.

Kemp has ruled out several hypotheses. He found that body size had no relation to victory. Then he measured various wing and body parameters to see whether flight dynamics could explain the oldsters' prowess. He compared wing area with body weight to see whether flying got easier. He weighed flight muscles and fat to see whether older males had more power or more energy in the bank. The answer is "no" to all of the above, Kemp reported in the July-August 2002 *Behavioral Ecology*.

Now, Kemp proposes his own hypothesis: The male that persists in the face of risks wins. Butterfly duels probably bring some low-level risks, such as wing rips from brushing a twig or general wear on the wings, he notes in the July 7, 2002 *Proceedings of the Royal Society of London B*. "A young guy has his whole life ahead of him," Kemp points out. He proposes that the older guy wins because he has less to lose.

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